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16. ABSTRACT

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Altered Function and Histology in Guinea Pigs after Inhalation of Diesel Exhaust

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Health effects of inhaled diesel engine exhaust were evaluated in infant guinea pigs following 4 and 8 weeks of exposure. Animals were exposed to 1 part exhaust diluted by 13 parts clean air for 20 hr/day, 7 days/week. Lung function, electrocardiogram, growth rate, and histopathology were assessed following exposure. After 4 weeks, animals exposed to irradiated exhaust, showed a 35% increase in pulmonary air flow resistance, and a small but significant decrease in heart rate. Necropsy, after 8 weeks, showed exhaust exposed animals to have black discoloration of the lungs and increased lung to body weight ratios. Microscopic examination of the lungs from these animals revealed black particulate material in the cytoplasm of alveolar macrophages and in draining lymph nodes.

INTRODUCTION

Diesel engine usage is expected to increase in the United States as a result of pressure exerted by fuel economy measures. Emissions from the diesel engine include both gaseous and particulate components with polynuclear aromatics adsorbed to the particulate. A number of studies have concluded that the exhaust particulate is potentially the greatest drawback of diesel-powered vehicles. Springer and Baines (1977) compared two diesel passenger cars with two emission-controlled gasoline-powered counterparts. The diesels emitted somewhat more hydrocarbons (HC), less carbon monoxide (CO), and essentially the same amount of nitrogen oxides (NOx) as did the gasoline cars; however, diesel cars emitted 54–82 times more particulate. Breslin *et al.* (1976) found the mass median diameters of diesel particulate to be typically about 0.3 μm. Furthermore, diameter was not dependent on engine type or fuels and only slightly dependent on operating mode. They concluded that essentially all diesel exhaust particulate can be considered respirable. Diesel exhaust has been characterized (Springer and Baines, 1977; Karasek *et al.*, 1974; Frey and Corn, 1967; Kotin *et al.*, 1955) and there is health effects information available on many of the individual components but there is very limited toxicological data on diesel exhaust per se. Because known carcinogens are present in the exhaust and have been extracted from diesel particulate, most health effects research has emphasized cancer risk (Mittler and Nicholson, 1957; Kotin *et al.*, 1955). A few studies have been conducted relating diesel exhaust exposure and pulmonary function in humans (Jorgenson and

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Svensson, 1970; Battigelli, 1965; Battigelli *et al.*, 1964) and in animals (Battigelli *et al.*, 1966; Pattle *et al.*, 1957). Human studies failed to show an adverse effect from diesel exposure and the few animal studies were inconclusive. Battigelli *et al.* (1966) found that tracheal clearance in rats was affected by diesel exhaust at concentrations that were without measurable effect on airway resistance in humans. The effect was attributed primarily to diesel particulate. Pattle *et al.* (1957) used undiluted exhaust and concluded that related animal deaths were caused by high levels of NO_2, CO, and acrolein-type irritants present in the emissions.

The objective of the present experiment was to evaluate the effects of inhaled diesel exhaust on pulmonary function, growth rate, electrocardiogram (ECG), and histology of tissues in guinea pigs exposed to exhaust. Pulmonary function and ECG were measured in unanesthetized animals following 1 month continuous exposure to diluted irradiated exhaust (IE) and clean air (CA) atmospheres. Growth rates of animals were computed and tissues were examined by light microscope after 2 months exposure to IE, CA, and diluted raw exhaust (RE) atmospheres.

MATERIALS AND METHODS

Exposure Conditions

Diesel exhaust was generated by a Nissan CN6-33 engine coupled to a Chrysler[3] torque-flite automatic transmission, Model A-727. No. 2 diesel fuel, typically used for automobiles with diesel engines, was chosen for this study. The dynamometer driving schedule used for the engine was a modified "California Cycle" (*Federal Register*, 1966, 31 (61), paragraph 85.72). Emissions from the engine were diluted 1:13 with clean air and introduced into animal exposure chambers as raw exhaust (RE) or into large chambers containing artificial sunlight and then to exposure chambers as irradiated exhaust. The average irradiation time was approximately 1 hr, during which photochemical reactions occurred producing a simulated "photochemical smog." The test atmospheres, RE, IE, and clean filtered air, were supplied to 100 ft^3 (2.83 m^3) animal-exposure chambers at 25°C and 50% relative humidity. The rate of flow yielded 15 air changes per hour. Details of this system have been presented elsewhere (Moore *et al.*, 1978; Burkart *et al.*, 1974; Hinners *et al.*, 1966, 1968). Analytical methods utilized for characterization of atmospheres in the animal exposure chambers have been described in previous studies (Cooper *et al.*, 1977; Hysell *et al.*, 1975). There were nine exposure chambers; three contained CA, three RE, and three IE.

One hundred and sixty Hartley strain guinea pigs [Crl:COBS (HA)], both male and female, were weaned at 4 days of age, numbered with ear tags, and randomly distributed between the three atmospheres where they remained throughout the study. Guinea pigs used for pulmonary and ECG studies were exposed continuously for 28 days (20 hr/day, 7 days/week) to IE and CA. Those examined for histological change were similarly exposed for 56 days to RE, IE, and CA. All animals were included in growth rate analysis. Due to time constraints on the

[3] No mention of a product or company name constitutes endorsement by the U.S. Environmental Protection Agency.

availability of exhaust atmospheres, only a total of 37 animals could be measured for pulmonary function and ECG. IE-exposed were arbitrarily chosen as the test group. Cage cleaning and feeding took place during the 4 hr periods when the engine was not in operation.

Animal Measurement

The method used for evaluation of the mechanical properties of lungs of unanesthetized guinea pigs was based on that developed by Amdur and Mead in 1958. Competence of pulmonary function was determined by means of two calculated parameters: airway resistance and lung compliance. Computation of resistance and compliance required simultaneous recordings of three variables: tidal volume, air flow in and out of the lungs, and intrapleural pressure. All recording was done on a Grass 7B polygraph. For measurement of tidal volume, the guinea pig was sealed inside a body plethysmograph. Volume changes inside the plethysmograph created by the animals' respiration were sensed by a Model PT5 volumetric pressure transducer (Grass Instruments). Calibration of the volume transducer was accomplished by means of a water-filled balloon placed inside the sealed plethysmograph connected to a syringe outside. The resulting calibration curve accommodated guinea pigs between 150 and 1200 g.

Flow was measured by electrical differentiation of the tidal volume signal with respect to time. Respiration rate and minute volume were read from the tracing. Intrapleural pressure was sensed via a saline-filled catheter, inserted into the chest cavity. A Statham P23Db pressure transducer, calibrated with a water manometer, was used for this measurement. The catheter was inserted into the intrapleural space under sterile surgical conditions 1 day prior to testing. The catheter was made of 18 cm of Dow Corning Silastic Medical Grade tubing (ID 0.030; OD 0.065 cm) open at one end and plugged at the other. Guinea pigs were anesthetized with sodium pentobarbital (30 mg/kg body wt, ip), a 2-cm longitudinal incision was made in the skin of the back just inferior to the scapula, and the open end of the catheter (a 2.5-cm segment) was inserted through the intercostal muscle into the intrapleural space. The catheter was secured to chest muscles at the chest wall with 4-O braided silk suture. The free end of the catheter was directed under the back skin to the left side and exteriorized via a skin puncture. The skin incision was sutured and the catheter secured for a second time at the puncture hole. The distal end of the catheter was coiled and wrapped with plastic tape, and the hind feet were wrapped with adhesive tape to prevent the animal from disturbing the catheter. Following surgery, each animal was individually housed in a cage and returned to its respective exposure chamber. For measurement of pulmonary function, the catheterized animal was removed from its chamber, fitted with a foam rubber collar, and sealed, at the neck, inside the plethysmograph. Once the animal was in the plethysmograph, the catheter was occluded at the chest wall, 8 cm of the distal end of the catheter was removed, and its open end attached to the transducer orifice (located in side the plethysmograph). Saline (0.02 ml) was introduced into the lumen of the catheter and the plethysmograph closed. The plethysmograph was then attached to the test exposure chamber (equipment illustrated in Fig. 1) and the appropriate atmospheric mixture turned on.

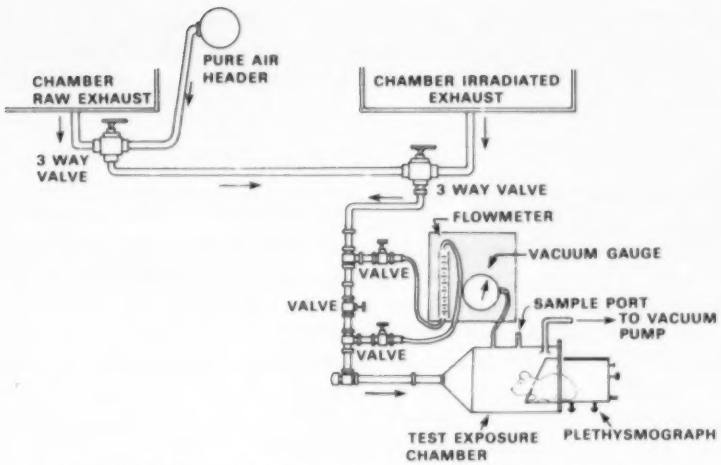

FIG. 1. Automobile exhaust gas exposure and measurement apparatus.

Placement of the animal in the plethysmograph required 5 min. The 28-day-exposed animal was prepared, placed in the plethysmograph–test chamber assembly and immediately equilibrated with its own exposure type atmosphere. Recordings of intrapleural pressure, tidal volume, flow, and the ECG were taken after 30, 45, and 60 min of equilibration. Tracings were taken during periods when the guinea pig was quiet and respiration was regular. Pulmonary resistance (R) and dynamic compliance (C) were computed from an equation described by Drazen *et al.* (1976) that relates the transpulmonary pressure (P) to tidal volume (V) and air flow ($\dot{V}$).

$$P = \frac{V}{C} + \dot{V}R. \tag{1}$$

Compliance is computed during tidal breathing at the instant of maximum volume and zero flow:

$$C = \frac{V}{P}, \qquad \dot{V} = 0. \tag{2}$$

Substituting the value of calculated C into Eq. (1), the resistance is computed at the instant of maximum flow:

$$R = \frac{P - V/C}{\dot{V}}, \qquad \dot{V} = \max. \tag{3}$$

Resistance and compliance were computed on four consecutive breaths and averaged for each measurement period. Growth rates were determined for each group in the study by constructing regression lines from weekly weights.

Histopathology

Following 56 days of exposure, 10 animals from each exposure atmosphere were anesthetized with pentobarbital for necropsy. Organs were removed, weighed, and stored in fresh phosphate-buffered formalin. Lungs and trachea were removed and the lungs inflated with 10% buffered formalin at 30 cm H_2O pressure, then tied. Gross lesions if any, were recorded, tissues were trimmed, processed, embedded in paraplast, cut at 4 to 6 μm, and stained with hematoxylin and eosin. Tissues examined by light microscopy were: lungs, (left apical and cardiac, left diaphragmatic and accessory, right apical and cardiac, right diaphragmatic and intermediate lobes), trachea and bronchial lymph nodes, adrenals, brain, colon, duodenum, gall bladder, heart, kidneys, liver, pancreas, testicles, uterus, and spleen.

Statistical Analysis

Physiological data were subjected to a two-way analysis of variance (Hays, 1963). Where significant F ratios were obtained differences between exposure group means were compared using a two-tailed Student's t test for unpaired data.

RESULTS

Aerometry

Mean concentrations of measured pollutants for the entire exposure period are given in Table 1. A scanning electron micrograph (SEM) of the diesel particulate is shown in Fig. 2. Particulate samples were collected on Nucleopore membranes from animal exposure chamber atmospheres. A circular piece was cut from each membrane and mounted on the SEM sample stub with conductive paint and overcoated with an approximately 200-Å-thick layer of gold–palladium alloy. The particulate appeared as aggregates and as extremely small individual particles resembling spherical cotton balls in the photomicrographs. All of the particulate in this photomicrograph was within the respirable range.

TABLE 1
CONCENTRATIONS OF MEASURED EXHAUST CONSTITUENTS INSIDE EXPOSURE CHAMBERS

	Clean air	Raw exhaust	Irradiated exhaust
Carbon dioxide (%)	0.04	0.26 ± 0.01	0.25 ± 0.03
Carbon monoxide (ppm)	2.0	17.4 ± 2.5	16.7 ± 4.0
Total hydrocarbons (ppm-C)	0.0	31.6 ± 2.3	26.1 ± 1.6
Nitric oxide (ppm)	0.11	5.9 ± 0.6	5.0 ± 1.2
Nitrogen dioxide (ppm)	0.07	2.3 ± 0.4	2.9 ± 0.7
Sulfur dioxide (ppm)	0.0	2.1 ± 0.8	1.9 ± 0.8
Ozone (ppm)	0.0	0.0	<0.01
Particulate			
Mass (mg/m³)	0.0	6.32 ± 1.31	6.83 ± 1.44
Sulfate (mg/m³)	0.0	0.57 ± 0.12	0.57 ± 0.13

[a] Values are means ± SD.

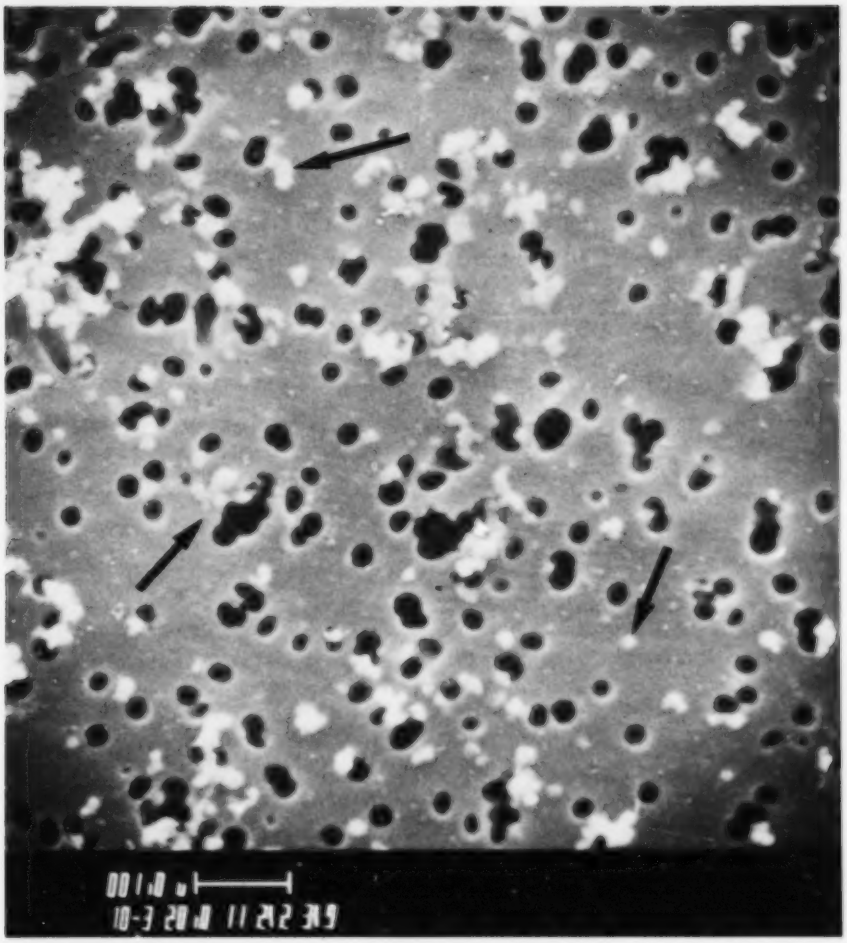

FIG. 2. A scanning electron micrograph of diesel particulate, collected from an animal chamber atmosphere. The particulate (arrows) is present in individual particles and in aggregates. Particulate shown here is in the respirable range.

Clinical Observations

No gross changes in general appearance or condition were observed in any of the guinea pigs during exposure. There was no mortality related to the exposure atmospheres.

Physiological Measurements

Growth of animals located in exhaust atmospheres was not different from that of the clean air group. CA, IE, and RE animals had linear growth rates of 7.58,

7.51, and 7.56 g per day, respectively, as seen in Table 2. Animals from IE atmospheres along with CA-exposed animals were selected for physiological testing. Pulmonary function values and heart rate measurements are shown in Table 3.

Pulmonary flow resistance was found to be increased ($P < 0.002$) in animals that had been exposed to IE for 28 days as compared to those exposed only to clean air. This increase was noted in measurements taken at 30, 45, and 60 min during equilibration with the exhaust atmosphere. Values for dynamic compliance, minute volume, respiration rate, and tidal volume showed no differences at any equilibration period between control and IE-exposed animals. Electrocardiograms were recorded on each animal during the pulmonary function testing periods. Lead I was used routinely. In almost all instances the animals were quiet while confined in the plethysmograph. The ECG pattern displayed positive P, R, and T waves and negative Q and S waves. Intervals between waves were not measured in detail for these animals; however, no abnormalities were noted in impulse formation, conduction, or repolarization for any animal. Guinea pigs exposed to IE developed a small but significant ($P < 0.002$) sinus bradycardia. This was true for each equilibration period.

Histopathology

The lung weight to body weight ratio was significantly increased in the exhaust-exposed animals as compared with clean air exposed. Lung weights of clean air animals were 0.68% of body weight, 0.78% for irradiated exhaust, and 0.82% for raw exhaust, as seen in Table 4. Heart weight ratios, however, were not different for the three groups.

Histological findings in clean air animals were limited and insignificant. Rare single alveolar macrophages with pink cytoplasm were present in the lungs of all guinea pigs. No sex-related differences were noted. Formalin-fixed lungs from diesel-exposed animals were uniform grey-black. Histologically no sex-related differences were noted and significant findings were restricted to the respiratory system. In addition no differences could be detected between animals exposed to IE or RE. Exposure of the guinea pig to diesel exhaust was characterized microscopically by a marked accumulation of black pigment-laden macrophages

TABLE 2
COMPARISON OF GROWTH RATE OF GUINEA PIGS EXPOSED TO CLEAN AIR AND TO THOSE EXPOSED TO DIESEL EXHAUST[a]

Exposure type	No. of guinea pigs	(growth/day)	Correlation[b]
Clean air	44	7.58 ± 1.64	0.99 ± 0.009
Irradiated exhaust	51	7.51 ± 1.26	0.99 ± 0.009
$P <$		NS	NS
Raw Exhaust	41	7.56 ± 1.05	0.99 ± 0.005
$P <$		NS	NS

[a] All values are means ± SD. NS, no significant difference.
[b] Test for linearity of growth.

TABLE 3

RESPONSE OF GUINEA PIGS TO 4 WEEKS OF EXPOSURE TO IRRADIATED DIESEL EXHAUST[a]

Equilibration time (min)	No. of guinea pigs	Resistance (cm H_2O/ml/sec)	Compliance (ml/cm H_2O)	Minute volume (ml/min)	Breathing rate (breaths/min)	Tidal volume (ml/breath)	Heart rate (beats/min)
30							
CA	18	0.22 ± 0.006	0.36 ± 0.14	171 ± 48	95 ± 18	1.82 ± 0.48	409 ± 22
IE	19	0.31 ± 0.11	0.37 ± 0.12	175 ± 48	88 ± 15	1.99 ± 0.57	384 ± 23
$P<$		0.002	NS	NS	NS	NS	0.002
45							
CA	18	0.25 ± 0.06	0.36 ± 0.13	153 ± 37	91 ± 13	1.70 ± 0.39	406 ± 24
IE	19	0.33 ± 0.08	0.35 ± 0.10	175 ± 66	89 ± 17	2.03 ± 0.72	381 ± 20
$P<$		0.002	NS	NS	NS	NS	0.002
60							
CA	18	0.24 ± 0.05	0.34 ± 0.12	155 ± 39	90 ± 15	1.74 ± 0.47	414 ± 21
IE	19	0.32 ± 0.08	0.34 ± 0.11	156 ± 47	84 ± 15	1.91 ± 0.62	381 ± 24
$P<$		0.002	NS	NS	NS	NS	0.002

[a] All values are means + SD. NS, no significant difference.

TABLE 4
COMPARISON OF LUNG AND HEART WET WEIGHTS FROM CLEAN AIR- AND DIESEL EXHAUST-EXPOSED GUINEA PIGS[a]

	No. of animals	Lung (% body wt)	Heart (% body wt)
Clean air	9	0.68 ± 0.06	0.37 ± 0.04
Irradiated exhaust	10	0.78 ± 0.05	0.35 ± 0.04
$P<$		0.002	NS
Raw exhaust	8	0.82 ± 0.13	0.38 ± 0.07
$P<$		0.02	NS

[a] All values are means ± SD. NS, no significant difference from clean air.

throughout the lung. In an occasional animal, slight localization of macrophages around blood vessels and respiratory bronchioles was noted. About 1–20 macrophages were noted in affected alveoli. Where pigment was slightly dispersed within the macrophage cytoplasm, it could be seen as fine black particles. In addition pigmented macrophages often mixed with a pink-blue mucinous material and desquamated epithelial cells occurred singly or in aggregates adherent to the mucosa or free within the lumen of the tracheobronchial tree. Occasional pigment granules were noticed within the mucosa of the tracheobronchial tree. Often goblet cells in the trachea, bronchi, and bronchioles were hypertrophied and distended with a light grey-blue mucin. No attempt was made to quantify goblet cell hypertrophy. In an occasional focal alveolus where larger numbers of pigmented macrophages were accumulated, hyperplasia of alveolar lining cells and a slight focal thickening of the interstitium was noted. In one animal (IE) large numbers of pigmented macrophages formed a coalescent aggregate surrounded by hypertrophied alveolar lining cells. Slight to moderate accumulation of black-pigmented macrophages was noted in cortical and medullary areas of peribronchial lymphoid nodules and draining bronchial and carinal lymph nodes. In a small bronchiole from one animal (RE) a sheet of cells filled the lumen, suggesting bronchiolitis obliterans, however, pulmonary tissue in the immediate area was not atelectatic. In all lungs examined, no evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.[4]

DISCUSSION

In this study, guinea pigs exposed to diluted irradiated diesel exhaust 20 hr each day for 28 days showed measureable pulmonary and heart rate effects attributable to the exhaust. Pulmonary flow resistance of these animals had increased 35% over that of animals exposed to clean air. No differences were noted in dynamic compliance, minute volume, respiratory rate, or tidal volume. Examination of electrocardiogram tracings revealed no exhaust-related changes with the exception of rate. There was a significant sinus bradycardia found in guinea pigs exposed to the exhaust when compared to those exposed to clean air. There were no

[4] Slides, microscopic examination, and interpretations were made by Robert W. Fleischman D.V.M., E.G. & G. Mason Research Institute, 57 Union Street, Worcester, Mass. 01608.

significant differences in growth rate between CA, IE, and RE groups. At autopsy, the lungs from IE- and RE-treated animals showed grey to black discoloration and lung weight to body weight ratios were significantly increased over that of CA-exposed animals. Large deposits of black diesel particulate were seen microscopically in the lungs of animals exposed to exhaust which may have accounted for the increased lung weights.

Studies in environmental toxicology should in some way be relatable to actual or potential exposure conditions, particularly human exposure conditions. Particulate matter generated by the diesel engine was the atmospheric component of most concern in this study. Particulate concentrations in both IE and RE chambers were 6.8 and 6.3 mg/m^3, respectively, and particles were found to be in a respirable size range (0.1–1 μm). Measurements made by Waller *et al.* (1961) showed particulate (smoke) levels in a road tunnel to reach peak levels of 3.2 mg/m^3. Smoke concentrations roughly correlated with the density of diesel-powered traffic. Battigelli *et al.* (1966) and Battigelli (1965) conducted studies using diesel exhaust dilutions geared to encompass the "worst level" found in diesel shops. Concentrations of exhaust pollutants in their experiments were comparable to those used in this study. Thus diesel particulate concentrations in the present study are high, however, somewhat representative of short-term environmental and occupational levels experienced by man.

Gaseous contaminants, CO, NOx, CO_2, SOx, and light hydrocarbons present in this study were beneath concentration levels at which overt *in vivo* responses in laboratory animals can be easily defined. Diesel particulate, on the other hand, has been shown by Battigelli *et al.* (1966) to be the most noxious agent in the mixture. Diesel particulate emitted into chambers of this study contained a substantial quantity of products derived from incomplete fuel combustion. At ambient chamber temperatures approximately one-half of the hydrocarbons (THC, Table 1) were adsorbed to particulate. In addition, small quantities of NOx and SOx were also adsorbed. Condensation of substances onto a particle greatly increases the local concentration of each adsorbed substance. It seems logical that if these "respirable packets" of highly concentrated irritable substances are inhaled and deposited on membranes in the trachea, bronchi, and alveoli, irritation might occur at these foci before adequate defense mechanisms could respond.

Guinea pigs exposed to diesel exhaust showed increased accumulation of black-pigmented macrophages in the alveoli. This increase in macrophages is a common response to pulmonary irritation and may reflect increased mobilization, increased cell adherence, and/or decreased removal of macrophages by the mucociliary blanket, lymphatics, and blood (Frost *et al.*, 1973; Brain, 1970). Granules of black pigment were also noted in focal areas of the tracheobronchial tree and in draining bronchial and carinal lymph nodes. Hypertrophy of goblet cells, seen in the tracheobronchial tree, presumably represent the response of these cells to pulmonary irritation from diesel exhaust. Battigelli *et al.* (1966) reported a general decrease in mucociliary clearance rates in rats after a 4-hr exposure to diesel exhaust using pollutant levels comparable to those in this study. Focal hyperplasia of alveolar lining cells, seen in the present study, presumably Type II granular pneumocytes, was noted which might have represented an early response of the alveolar wall to injury by diesel exhaust.

Suckling rats simultaneously exposed under identical conditions as the guinea pigs showed black-pigmented macrophages after only 8 hr of exposure (Moore *et al.*, 1978), however no pigment could be found 28 days after the animals had been removed from the exhaust. Those exposed to diesel exhaust 8 hr per day for 5 consecutive days demonstrated pigmented macrophages 28 days following exposure and those exposed 20 hr per day for 16 days still retained pigment-laden macrophages after 90 days in clean air. Thus diesel particulate appears to have a long residence time in the lung permitting an extended period for elution of chemicals from the particles.

Other exposure studies in animals that might be compared to diesel emission exposure are those where health effects of carbon dust from furnaces or carbon dust combined with irritant gases have been observed. Nau *et al.* (1962) exposed hamsters, mice, guinea pigs, rabbits, and monkeys to channel black (85 mg/m^3, 25 nm diameter; no benzene extractible)[5] and furnace black (56 mg/m^3, 35 nm diameter; 0.28% benzene extractible)[5] 7 hr/day, 5 days/week for varying periods of time. Electrocardiograms from monkeys exposed for 1000 hr showed evidence of right atrial and right ventricular strain which increased in severity for up to 10,000 hr of exposure. Mild bradycardia, following 560 hr of exposure to IE was observed in the present study. Nau *et al.* (1962) also found, in mice, that after 1000 hr of exposure the ratio of heart weight to body weight was slightly increased. This was not shown to be true for our guinea pigs after 1120 hr of exposure to either IE or RE atmospheres. In addition, no abnormalities in heart tissue were noted microscopically in our treated animals, thus mechanisms responsible for the bradycardia are not yet clearly defined.

Wehr *et al.* (1975), in a study involving humans, stated that prolonged inhalation of activated carbon dust led to pulmonary deposition and signs of pneumoconiosis but had little if any effect on the respiratory symptoms or pulmonary function. Nau *et al.* (1962) found an increase in the lung weight to body weight ratio in their exposed animals; this was also found to be true of exhaust-treated animals in our study. They also found that microscopic changes were confined primarily to carbon deposition in the lungs. In contrast to this, histologic evidence of irritant response to diesel atmospheres in the tracheobronchial tree and alveoli was apparent in the present investigation. This finding parallels the significant increase in airway resistance seen in IE animals. Airway resistance, as measured in these guinea pigs, chiefly represents resistance to airflow in the larger proximal airways (i.e., the trachea and main bronchi). An increase in resistance values for a test group compared to a control group indicates a narrowing of the lumen of these airways or an increase in airflow turbulence therein. Since the guinea pigs were exposed continuously to test atmospheres for 1 month and pulmonary function was measured while the animals were equilibrated with familiar atmospheres it is unlikely that the increase in airway resistance was mediated via the vagus by contraction of bronchoconstrictor muscles, as described by Nadel *et al.* (1965). The increase in resistance probably reflected airway narrowing due to deposits of

[5] Correct values: Quantities cited in the original article were in error. Corrections in "Criteria for a Recommended Standard . . . Occupational Exposure to Carbon Black" (1978), U.S. DHEW (NIOSH) Publication No. 78-204, pp. 25–47.

diesel particles in the tracheobronchial tree with subsequent tissue reaction, and possibly, increased flow turbulence due to exhaust-related irregularities on luminal surfaces. Whether or not these functional changes would persist in the absence of inhaled irradiated exhaust was not determined in this study. However, if the guinea pig response to diesel exhaust was similar to rats (Moore *et al.*, 1978), significant deposits of diesel particulate would remain in the lungs for many months.

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